

A four-legged snake from the Early Cretaceous of Gondwana

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Snakes are a remarkably diverse and successful group today, but their evolutionary origins are obscure. The discovery of snakes with two legs has shed light on the transition from lizards to snakes, but no snake has been described with four limbs, and the ecology of early snakes is poorly known. We describe a four-limbed snake from the Early Cretaceous (Aptian) Crato Formation of Brazil. The snake has a serpentiform body plan with an elongate trunk, short tail, and large ventral scales suggesting characteristic serpentine locomotion, yet retains small, prehensile limbs. Skull and body proportions as well as reduced neural spines indicate fossorial adaptation, suggesting that snakes evolved from burrowing rather than marine ancestors. Hooked teeth, an intramandibular joint, a flexible spine capable of constricting prey, and the presence of vertebrate remains in the guts indicate that this species preyed on vertebrates, and that snakes made the transition to carnivory early in their history. The structure of the hind limbs suggests that they were adapted for grasping, either to seize prey or as claspers during mating. Together with a diverse fauna of basal snakes from the Cretaceous of South America, Africa, and India, this snake suggests that crown Serpentes originated in Gondwana.

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Snakes are among the most diverse groups of tetrapods, with >3,000 extant species exploiting a remarkable range of niches(1). Snakes inhabit deserts and rainforests, mountains and oceans, and despite lacking limbs, employ an extraordinary range of locomotor styles, including crawling, burrowing, climbing, swimming, and even gliding(1). All snakes are predators, but they consume a wide range of prey, from insects to large mammals (1). This diversity is made possible by a specialized body plan, including an elongate body with reduced limbs, a flexible skull and ribs to swallow large prey(2), and a specialized forked tongue and vomeronasal organ to detect chemical gradients(1). The origins of this body plan remain unclear, however(1). One scenario holds that this unique body plan originated in a marine environment, while others argue that it results from adaptation for a fossorial lifestyle. New fossils(2-4), including snakes with hindlimbs(5, 6) have shed light on the lizard-snake transition, but no snake has been reported with four limbs. The ecology of early snakes is also uncertain. Although alethinophidians are primarily carnivorous (1), Typhlopidae and Anomalepididae, which are basal with respect to Alethinophidia (7-9), are insectivorous (1). This suggests that early snakes were insectivores, however adaptations for carnivory in stem snakes(2), suggest that carnivory may be primitive (2, 5).

Here, we report a fossil snake from the Early Cretaceous of Gondwana which sheds light on these issues. *Tetrapodophis amplexus* (gen. et sp. nov.) is distinguished from all known snakes in retaining four limbs, displaying a unique manual and pedal morphology with shortened metapodials, hyper-elongate and curved penultimate phalanges, and a digital formula of 2-3-3-3-3.

The fossil (Fig. 1) comes from the Nova Olinda member of the Early Cretaceous (Aptian) Crato Formation, Ceará, Brazil(10). The specimen is preserved on laminated limestone as part and counterpart. The matrix is typical of the Nova Olinda member of the Crato Formation in being composed of fine-grained, laminated micrite with elongated pellets on the surface of the slab representing coprolites of the fish *Dastilbe*. As is typical of Crato vertebrates, the skeleton is articulated and the bones are a translucent orange-brown color; soft tissues are also preserved.

The snake affinities of *Tetrapodophis* are demonstrated by derived features of the skull, axial skeleton, limbs, integument and even behavior (* = snake autapomorphy). Snake-like features of the skull include a short rostrum, a long braincase, and a nasal descending lamina. The mandible is bowed, with a deep subdental ridge, and an intramandibular joint formed by a concave splenial cotyle contacting the angular, as in *Dinilysia*(11). Teeth exhibit the ophidian condition, being unicuspid and hooked, with expanded bases. Implantation is subacrodont implantation, with teeth separated by interdental ridges; replacement teeth are oriented subhorizontally*. Snake-like features of the axial skeleton include an elongate trunk with over 150 vertebrae*, zygosphen-zygantrum articulations; an arched neural arch with posterolateral tuberosities*; short neural spines; haemal keels; large subcentral fossae/foramina, tubercular processes of the ribs, and lymphapophyses. The ilium is long and slender as in other snakes; the fibula is bowed as in *Najash*(5) and Simoliophiidae(12). Transverse belly scales* are preserved, and the presence of a vertebrate in the gut suggests a snake-like feeding strategy in which proportionately large prey are ingested whole. While many of these features occur in other long-bodied squamates, only snakes exhibit all of them, and many of these characters are uniquely ophidian.

Tetrapodophis exhibits a number of primitive characters, however. The nasal has a distinctive L-shape, as in *Dinilysia*(11) and Simoliophiidae. The facial process of the maxilla is reduced compared to lizards but tall relative to crown snakes, as in *Coniophis*(2). The subdental ridge is shallow posteriorly, a primitive feature shared with *Najash*(5) and *Coniophis*(2). Unlike crown snakes, where a convex splenial condyle articulates with the angular, the splenial exhibits a concave cotyle, as in *Dinilysia*(11). Prezygapophyseal processes are absent as in other stem snakes; synapophyses are kidney-shaped, lacking the distinct condyle and planar cotyle of alethinophidians. Strikingly, *Tetrapodophis* retains reduced but apparently functional forelimbs and hindlimbs.

To test *Tetrapodophis*' ophidian affinities we used a morphological matrix(13, 14) to conduct four phylogenetic analyses: with and without molecular backbone constraint(8), and with equal and implied weighting(15). In each analysis, *Tetrapodophis* emerges as a basal snake. When a molecular backbone is used (Fig. 5), *Tetrapodophis*

emerges as sister to *Coniophis*, and snakes emerge as sister to the Mosasauria, i.e. Pythonomorpha, as in a recent combined analysis(7).

As the only known four-legged snake, *Tetrapodophis* sheds light on the evolution of snakes from lizards. *Tetrapodophis* lacks aquatic adaptations (e.g. pachyostosis, long, laterally compressed tail) and instead exhibits features of fossorial snakes and lizards: a short rostrum and elongation of the postorbital skull, a long trunk and short tail(16, 17), short neural spines(18), and highly reduced limbs(16, 17). *Tetrapodophis* therefore supports the hypothesis that snakes evolved from burrowing(2, 5, 6), rather than marine(19) ancestors. While the current analysis suggests a sister-group relationship between Mosasauria and Serpentes, Cretaceous aquatic snakes (Simoliophiidae) are recovered nested within crown Serpentes, aquatic habits are therefore derived within snakes(2, 7).

Tetrapodophis also sheds light on the evolution of snake feeding. *Tetrapodophis* exhibits adaptations for carnivory, including recurved, claw-like teeth to seize large prey, and an intramandibular joint allowing the gape to expand to swallow large prey. Along with the presence of a vertebrate in the gut, these features show that *Tetrapodophis* preyed on vertebrates. Similar adaptations occur in other early snakes(2, 11), suggesting that snakes made the transition to carnivory early in their history, and that the insectivorous lifestyle of typhlopids and anomalolepidids is derived.

The structure of the spine may represent another such adaptation for carnivory. Elongate bodies and reduced limbs evolved many times among squamates(13, 17), occurring in burrowing and terrestrial forms(17), as well as aquatic mosasaurs. Yet snakes are unique among long-bodied squamates in having over 150 precaudal vertebrae. This permits extreme flexibility of the spinal column, such that the entire body can coil into tight loops. The fact that other long-bodied squamates lack this feature suggests that it is not related to locomotion. We propose that the increased number of trunk vertebrae may be an adaptation allowing the body to be used to constrict prey. *Tetrapodophis* exhibits both an increased number of precaudal vertebrae and a high degree of flexibility, with the body forming a tight coil anteriorly and a series of sinuous curves posteriorly, suggesting that constriction was developed even in the earliest snakes.

The structure of the limbs may represent another predatory adaptation. The snake-like spine and reduced limbs of *Tetrapodophis* suggest that the animal engaged in characteristic serpentine locomotion, with the limbs playing little or no role in locomotion. However, the specialized structure of the limbs suggests that they were functional. Given *Tetrapodophis*' presumed fossorial habits, digging is a plausible function, but the limbs lack fossorial specializations. Instead, the manus and pes exhibit slender, isodactyl digits with hyper-elongate penultimate phalanges and abbreviated proximal phalanges. This suite of characters recalls the prehensile feet of animals such as scansorial birds, sloths(20) and bats, suggesting a grasping or hooking function. Conceivably, the limbs could have functioned for grasping prey, or perhaps mates. Climbing is another possibility, although the low neural spines seem inconsistent with this function. Regardless, *Tetrapodophis* shows that after the initial evolution of serpentine locomotion, the limbs were repurposed for another function.

Finally, *Tetrapodophis* sheds light on the geographic origin of snakes. The Serpentes, Iguania, and Anguimorpha form the Toxicofera(7-9), with the oldest iguanian and anguimorph fossils coming from Laurasia(13). These patterns suggest the center of toxicoferan diversification is Laurasia, and that the ancestors of snakes probably originated there. The identification of possible stem ophidians from the Jurassic and Early Cretaceous of Laurasia(4) would support this hypothesis. However, the most basal divergences within crown Serpentes, including Anomalolepididae and Typhlopidae, Aniliidae and Tropidophiidae, are endemic to or originate in South America and Africa, hinting at Gondwana origins(1). Furthermore, during the middle Cretaceous, Gondwana was home to a diverse fauna of basal snakes, including Coniophiidae, Russellophiidae, Madtsoiidae(21), and Simoliophiidae(22) in the Cenomanian of Africa, the Cenomanian-Turonian *Najash*(18, 23) in South America, and now *Tetrapodophis* from the Aptian of South America. Snakes are far less diverse in the Cretaceous of Laurasia, with a single lineage appearing in the Cenomanian(24) of North America; alethinophidians do not appear until the Maastrichtian in North America(14) and Europe(25). These patterns suggest that the Serpentes represent an endemic Gondwanan radiation that saw limited dispersal to Laurasia during the Cretaceous.

Snakes appear to have been part of a highly endemic herpetofauna that evolved in the Cretaceous in Gondwana. In this fauna, notosuchian crocodiles(26) and rhynchocephalians(27) played a major role, while squamates appear to have been less diverse and disparate than in Laurasia. The exception is the snakes, which radiated to produce small burrowers, large constrictors, and aquatic forms(21, 22). Much of this unique herpetofauna appears to have become extinct during the K-Pg extinction (Notosuchia) or was greatly reduced in diversity in the Cenozoic (Rhynchocephalia). Snakes, meanwhile, not only survived but became diverse and widespread in the Paleogene(14), perhaps in response to ecological release provided by the end-Cretaceous mass extinction(14).

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28. DM, NRL and HT designed and performed research; NRL performed the phylogenetic analysis, DM and NRL wrote the paper.

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SUPPLEMENTARY MATERIALS

Figs S1-S7, Table S1

SOM Text

Character-taxon matrix

Constraint tree



Fig. 1. *Tetrapodophis amplexus*, holotype part and counterpart. (A), counterpart, showing skull and skeleton impression, (B) main slab, showing skeleton and skull impression.

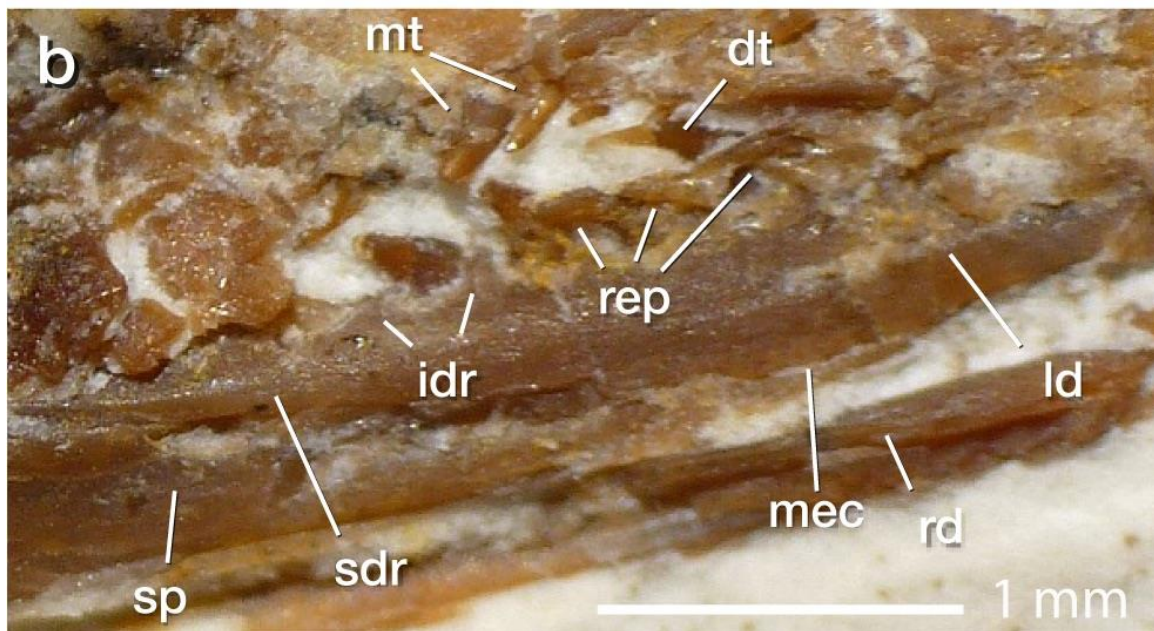
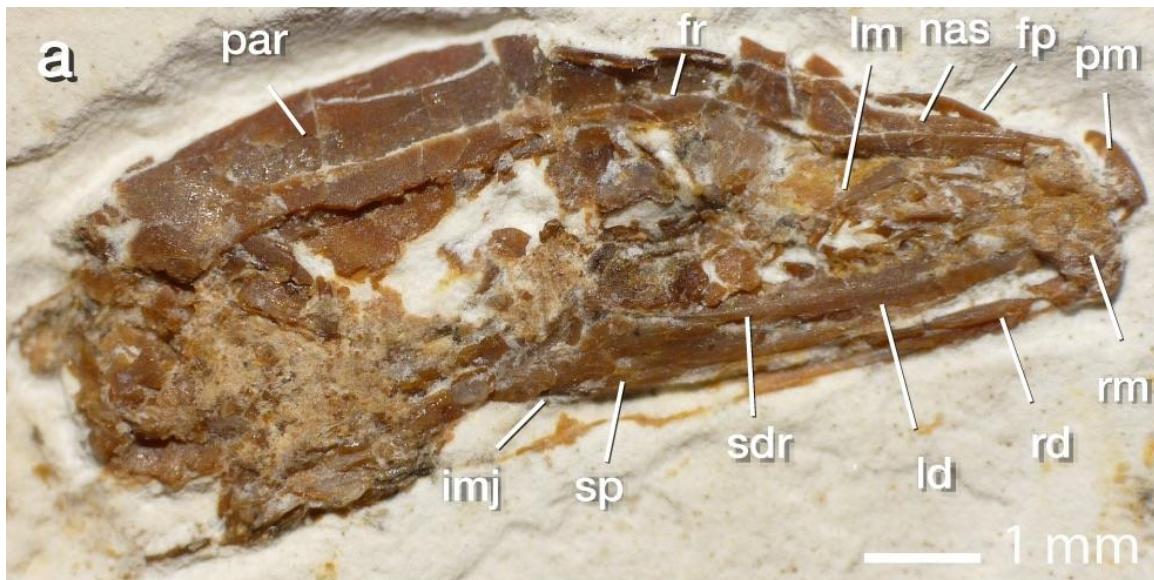


Fig. 2. *Tetrapodophis amplexus*, skull and jaws. (A) skull, (B) left mandible in medial view. Abbreviations: dt, dentary tooth; fp, facial process of maxilla, fr, frontal; lm, left maxilla, ld, left dentary; mt, maxillary teeth; nas, nasal, par, parietal; pm, premaxilla; rd, right dentary; rd, right dentary; rt, replacement teeth; sdr, subdental ridge; sp, splenial.

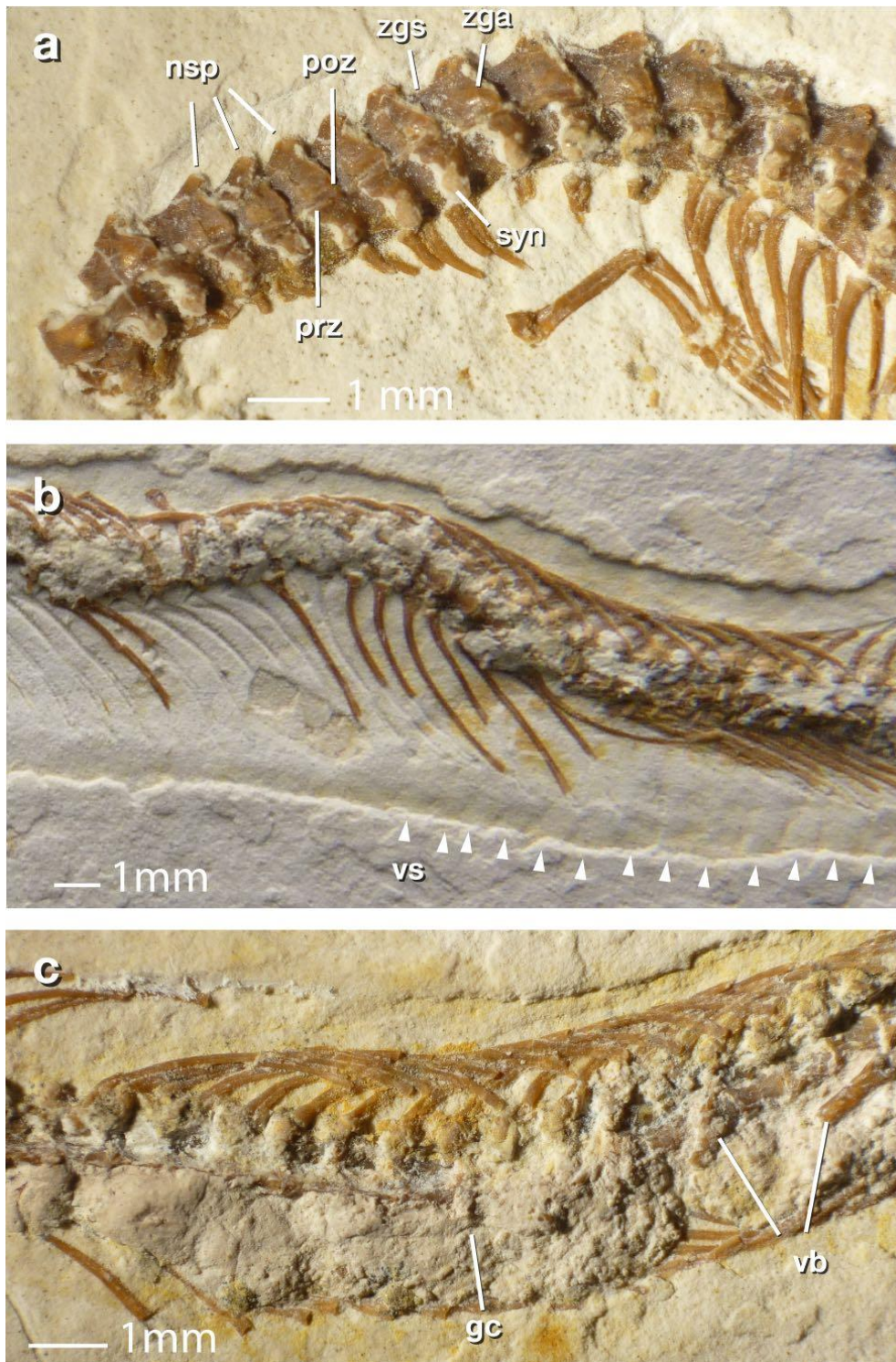


Fig. 3. *Tetrapodophis amplexus* axial column. (A) cervicals and anterior presacrals, (B) mid-thorax, showing ventral scales, (C) posterior thorax, showing gut contents. Abbreviations: gc, gut contents; nsp, neural spines; poz, postzygapophysis; prz, prezygapophysis; vb, vertebrate bone; vs, ventral scales; zga, zygantrum; zgs, zygosphenes.

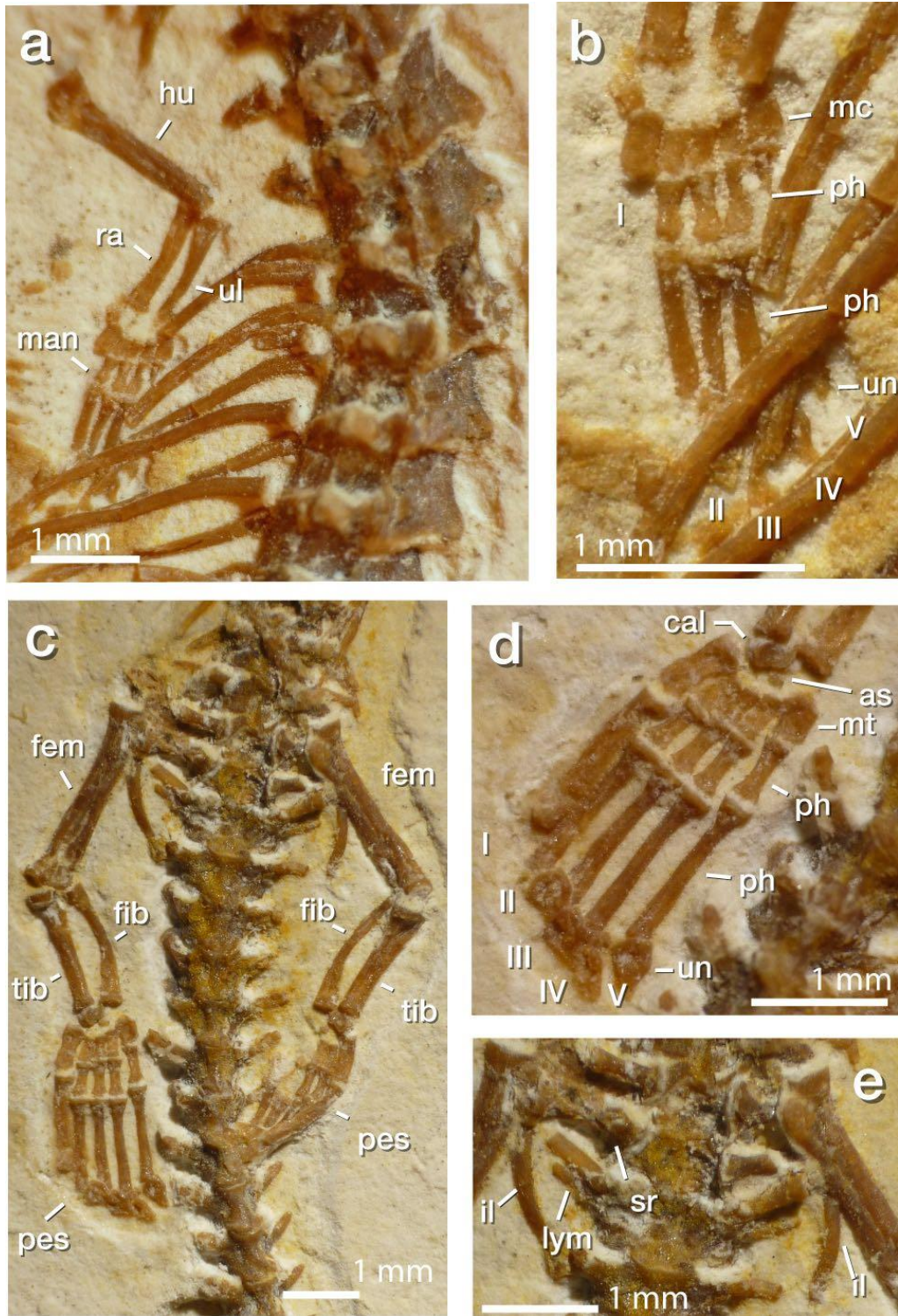


Fig. 4. *Tetrapodophis amplexus* appendicular morphology. (A) forelimb, (B) manus, (C) hindlimbs and pelvis, (D) pes, (D) pelvis. Abbreviations: fem, femur; fib, fibula; hu, humerus; il, ilium; lym, lymphapophysis, ma, manus; mc, metacarpal; mt, metatarsals; ph, phalanges; ra, radius; sr, sacral rib; tib, tibia; ul, ulna; un, ungual.

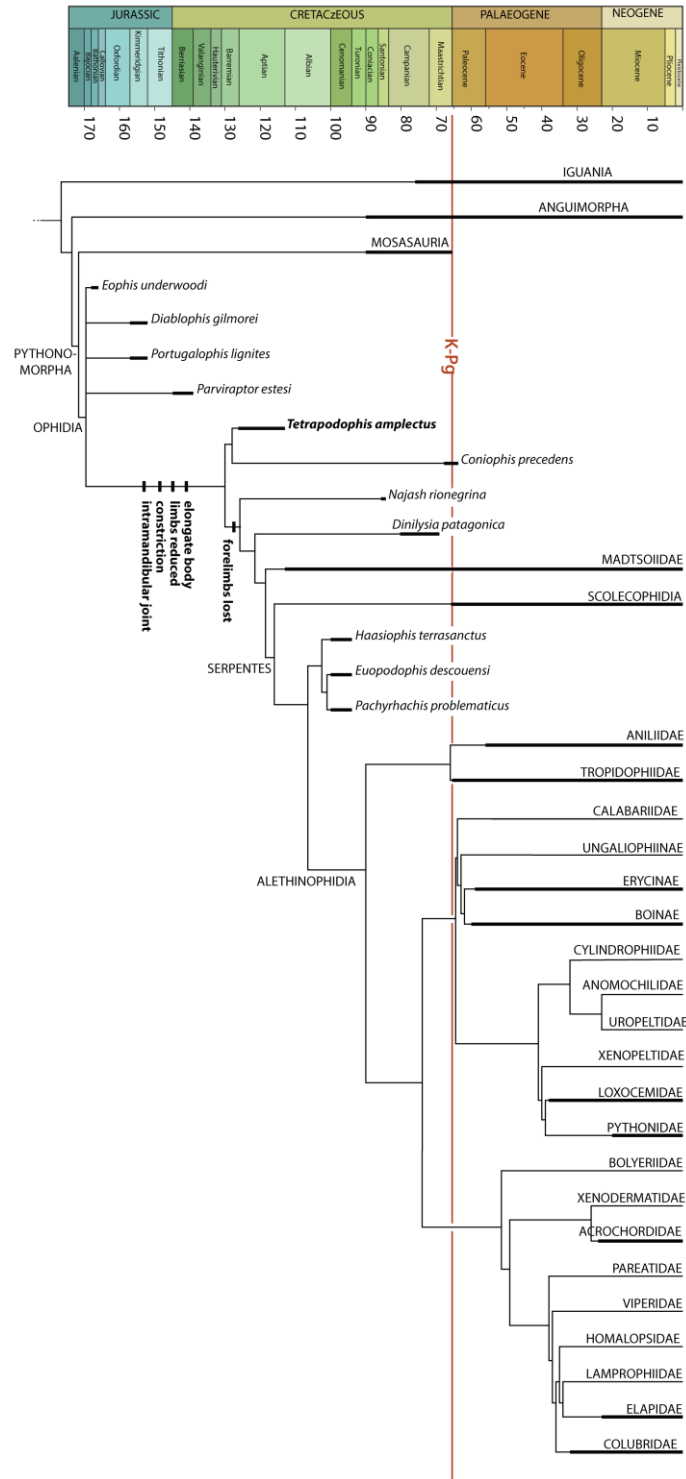


Fig. 5. Phylogenetic position of *Tetrapodophis amplexus*. Strict consensus of 85 most parsimonious trees found using implied weights and molecular constraint (see SI) for a matrix of 632 characters and 205 taxa.